Polymorphisms in cytokine genes define subpopulations of HIV-1 patients who experienced immune restoration diseases

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Objective: To further elucidate the immunopathogenesis of immune restoration diseases (IRD) in HIV patients responding to antiretroviral therapy and determine whether IRD associated with different opportunistic pathogens involve distinct immunopathological mechanisms.

Design: DNA samples from patients with a range of IRD were typed for polymorphic loci in genes encoding immune-mediators.

Methods: PCR–restriction fragment length polymorphism assays were used to type loci in the *IL1A, IL1B, IL6, TNFA* and *IL12B* genes. Alleles of a microsatellite in the CD30 promoter were determined by capillary electrophoresis.

Results: Only 8% of patients with IRD associated with a herpesvirus infection carried IL12B-3′UTR*2, compared with 42–54% of patients with other or no IRD. Patients with IRD arising from mycobacterial infection rarely carried IL6-174*C (36% versus 61–71%) and never carried TNFA-308*2 (0% versus 23–52%). TNFA-308*2 was carried by 52% of patients who experienced IRD associated with a herpesvirus infection, as several patients with exacerbations of cytomegalovirus retinitis carried this as part of a HLA-A2,B44 haplotype. Polymorphisms in *IL1A, IL1B* and *CD30* showed no distinct patterns.

Conclusions: Distinct cytokine-mediated mechanisms contribute to IRD initiated by herpesvirus and mycobacterial infections. © 2002 Lippincott Williams & Wilkins

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Introduction

Some HIV patients who respond to highly active antiretroviral therapy (HAART) experience atypical presentations of diseases arising from pre-existing (often quiescent) infections by opportunistic pathogens [1,2]. We have argued that these are immune restoration diseases (IRD), which result from an immunopathological response to the antigens of opportunistic pathogens [1,3]. This model was developed several years ago when we showed that disease associated with *Mycobacterium avium* complex (MAC) infection occurred after nucleoside analogue therapy and correlated with restoration of delayed hypersensitivity (DTH) responses to mycobacterial antigens [4]. More recently, clinical characteristics of IRD associated with infection by cytomegalovirus (CMV), MAC, *Mycobacterium tuberculosis* (MTB), varicella zoster virus (VZV), herpes simplex virus (HSV), hepatitis B virus (HBV) and hepatitis C virus (HCV) after HAART have been described (reviewed in [3]). CMV and HCV IRD correlate with increased antibody titres to these pathogens [5,6] and mycobacterial IRD correlate with increased DTH or lymphoproliferative responses to mycobacterial antigens [3,7], indicating immunological recognition of the pathogen, but the critical cytokines have not been identified.

Whilst IRD may resolve spontaneously, some result in significant morbidity and occasionally mortality. Examples include immune recovery uveitis in patients with previous CMV retinitis [8], suppurative lymphadenitis associated with MAC infection [9], rapidly progressive hepatitis associated with HCV [10] and inflammatory reactions in progressive multifocal leucoencephalopathy associated with JC virus [11]. It is therefore important to understand the pathogenic mechanisms so that approaches to the management of these diseases can be devised.

We have assembled a cohort of IRD patients for studies of immunological and genetic determinants of disease and showed that HLA-B44 and the major histocompatibilty complex (MHC) haplotype HLA-A2,B44,DR4 are relatively common in patients who experienced an IRD that manifested as CMV retinitis and/or encephalomyelitis associated with presumptive HSV infection [12]. Here we investigate polymorphisms in cytokine genes to determine whether the encoded cytokines affect the pathogenesis of IRD.

Materials and methods

Adult HIV-infected patients attending clinics at Royal Perth Hospital (Western Australia) were selected on the basis of an infectious/inflammatory disease after responding to antiretroviral therapy, mostly in the first 3 months of treatment (Table 1). Mycobacterial IRD were associated with cutaneous DTH responses to tuberculin and/or *M. avium* purified protein deriviative in all patients. Three patients with MAC disease had been treated with zidovudine monotherapy [4], but the remainder had received HAART (triple therapy including a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor). All three patients with Kaposi's sarcoma (KS) had a severe disease exacerbation or *de novo* presentation of KS lesions, which resolved without treatment, under observation in clinical trial protocols. Further details of all patients except those with KS are presented elsewhere [1,4,6,13]. A non-IRD control group was selected on the basis of a CD4 T-cell count $< 100 \times 10^6$/l before HAART and an increase of greater than fourfold or to $> 200 \times 10^6$/l CD4 T cells/l. Non-IRD controls were excluded if there was insufficient follow-up data, or if they had experienced undefined disease during immune reconstitution. An HIV-seronegative control group (n = 154) was obtained from the Western Australian Bone Marrow Donor Registry. The protocol was approved by the Ethics Committee of Royal Perth Hospital.

Genomic DNA was screened using PCR–restriction fragment length polymorphism assays as described to determine the alleles carried at interleukin (IL)6-174 [14], tumour necrosis factor alpha (TNFA)-308 [15],

Table 1. Immune restoration diseases (IRD) in the patient cohort.

	n
Patients with IRD associated with herpesvirus infection (n = 25)	
CMV retinitis alone	4
CMV retinitis and dermatomal zoster	1
Encephalomyelitis (associated with presumptive HSV infection) alone	2
Encephalomyelitis (associated with presumptive HSV infection) and MAC IRD[a]	1
Dermatomal zoster alone	8
Dermatomal zoster and Kaposi's sarcoma	2
Kaposi's sarcoma alone	1
Mucocutaneous HSV lesions	5
VZV encephalitis	1
HCV co-infected patients with > 3-fold rise in serum ALT after HAART (n = 13)	
With symptomatic hepatitis (as the only IRD)	3
With symptomatic hepatitis preceded by MAC IRD[a]	1
Without symptomatic hepatitis (as the only IRD)	9
Patients with IRD associated with mycobacterial infection (n = 11)	
MAC disease after zidovudine monotherapy	3
MAC disease after HAART (as the only IRD)	4
MAC disease after HAART followed by HCV IRD[a]	1
MAC disease after HAART preceded by encephalomyelitis[a]	1
MTB disease after HAART	2

[a]These patients are included in two groups. CMV, Cytomegalovirus; HSV, Herpes simplex virus; MAC, *Mycobacterium avium* complex; VZV, varicella zoster virus; HCV, hepatitis C virus; ALT, alanine aminotransferase; HAART, highly active antiretroviral therapy; MTB, *Mycobacterium tuberculosis*.

IL12B-3′UTR [16] and IL12B-promoter [17], and IL1A-889 and IL1B+3953 [18]. Homozygous and heterozygous samples were included in every run as controls. The C allele at IL6-174 yielded products of 122, 111 and 57 base pairs (bp), whilst the G allele yielded 233 and 57 bp bands. Allele 1 at TNFA-308 (G) yielded 87 and 20 bp products and allele 2 (A) gave a 107 bp band. Allele 1 (A) at IL12B-3′UTR (position 16974 of IL12B sequence; Genbank AY008847) yielded a 300 bp band and allele 2 (C) yielded bands of 166 and 134 bp. The *IL12B* promoter polymorphism was typed using the following primers (5′-TCAGA CACATTAACCTTGCA-3′ and 5′-TAATGTGGTC ATTGGCAGGT-3′ [17]). Allele 1 was designated as the 4 bp larger allele; the smaller product was designated as allele 2. IL1A-889 allele 1 (C) yielded 83 and 16 bp products, whilst allele 2 (T) yielded a 99 bp product. IL1B+3954 allele 1 (C) yielded 97 and 85 bp bands, whilst allele 2 (T) yielded a single band of 182 bp. A microsatellite in the promoter of the CD30 gene was typed by PCR amplification and sized relative to internal controls on an ABI310 Gene Scanner [19]. The predominant CD30 microsatellite alleles in HIV-seronegative controls were allele 358 (17%), allele 370 (17%), allele 374 (35%) and allele 378 (10%). The lowest and highest values were 342 and 404 bp, respectively. The results were also analysed as the percentage of patients carrying alleles of sizes above the 80th and below the 20th percentile for HIV-negative controls. Statistical analyses were based on Fisher's exact test as some cells contained fewer than five values. $P \leqslant 0.05$ was accepted as a significant difference. The results were not corrected for multiple comparisons as, in a first pass exploratory study, the inclusion of polymorphisms which did not affect outcome (e.g., IL1A-889 and IL1B+3953) should not determine whether the effects of other single nucleotide polymorphism (SNP) (e.g., TNFA-308) were significant.

Results

Alleles carried at IL1A-889 and IL1B+3953 were determined in a representative subset of the cohort. As no significant associations were evident, other samples were not tested (Table 2). TNFA-308*2 was carried by similar proportions of HIV-negative controls, non-IRD patients and HCV IRD patients, but was slightly more common in patients with herpesvirus-associated IRD (Fisher's exact test, $P = 0.042$ relative to patients without IRD) and was not present in any patients who had experienced IRD associated with MAC or MTB infection ($P = 0.035$ relative to patients without IRD; $P = 0.015$ relative to seronegative controls).

Carriage of the C allele at IL6-174 was common in patients and control subjects (61–74% carriage), but was found in only four of 11 patients (36%) who had IRD associated with mycobacterial infection ($P = 0.05$ relative to HIV-negative subjects). IRD associated with MAC or MTB infection occurred only in individuals who were homozygous for TNFA-308*1 (100% carriage), so carriage of TNFA-308*1 and non-carriage of IL6-174*C was clearly more common than in patients without IRD (relative risk = 3.0; $P = 0.014$).

All patients who experienced CMV retinitis, or encephalomyelitis associated with VZV infection or presumptive HSV infection, 10 of 11 patients with cutaneous VZV and/or KS, and four of five patients who experienced cutaneous HSV lesions as IRD were homozygous for IL12B-3′UTR*1, whilst patients who experienced IRD associated with HCV or mycobacterial infection had similar carriage rates to controls. Thus, carriage of allele 2 was less frequent in the combined herpesvirus-IRD group than seronegative controls [relative risk (RR), 0.29; $P = 0.028$] or non-IRD patients (RR, 0.21; $P = 0.0043$). Small differences were seen when carriage rates for the IL12B-promoter allele 2 in

Table 2. Immune restoration diseases (IRD) are associated with polymorphisms in the *TNFA, IL12B* and *IL6* genes. Results are presented as the percentage of individuals carrying the alleles shown.

	HIV-negative controls	HIV-positive controls without IRD	HIV patients who experienced herpes virus disease after HAART	HIV/HCV patients who experienced hepatotoxicity after HAART	HIV patients who experienced mycobacterial disease after ART
n	60	33	18	11	8
IL1A-889*2	45%	40%	53%	63%	75%
IL1B+3953*2	47%	36%	53%	36%	50%
n	100–154	33	25	13	11
IL6-174*C	66%	64%	71%	61%	36%[c]
TNFA-308*2	34%	24%	52%[b]	23%	0%[b,c]
IL12-3′UTR*2	31%[a]	42%	8%[b,c]	54%	50%
IL12-promoter*2	83%[a]	85%	86%	54%[b,c]	62%

[a] n = 100 HIV-negative controls. [b] Significantly different from patients with no IRD (Fisher's exact test, $P < 0.05$). [c] Significantly different from HIV-negative controls (Fishers exact test, $P < 0.05$). HCV, Hepatitis C virus; HAART, highly active antiretroviral therapy; ART, antiretroviral therapy.

HCV IRD patients were compared with seronegative controls (RR, 0.65; $P = 0.024$) and non-IRD patients (RR, 0.64; $P = 0.036$).

There were no clear differences in the frequency of carriage of any individual allele of the microsatellite in the *CD30* promoter. To reduce the numbers of categories, we also compared the percentage of patients carrying alleles of sizes above the 80th and below the 20th percentile with HIV-negative controls. High molecular weight alleles were more common in HCV IRD patients (RR, 2.5; $P = 0.03$ relative to seronegative controls), although several HCV patients carried the 378 bp allele which lies at the 80th percentile (data not shown). Further analyses will require a larger cohort.

Discussion

The decline in the incidence of opportunistic infections in HIV patients since the widespread use of HAART demonstrates that pathogen-specific immune responses can be restored. However, some patients experience atypical presentations of opportunistic infections despite a virological and immunological response to the therapy. We have defined them as IRD. Polymorphisms in cytokine genes may impact upon IRD if they influence the clearance of the opportunistic pathogen before or after HAART, or if they exacerbate an immunopathological response to the pathogen, manifested as an IRD. We favour the second model because both CMV and MAC IRD may occur in patients receiving anti-CMV or anti-MAC prophylactic therapy at the time of the IRD [1,20]. In addition, CMV, HCV and MAC IRD correlate with restored or increased immune responses to the initiating organisms [1,3,4–7,13]. Hence, we suggest that tissue inflammation arises from dysregulation in the restored immune response to the residual antigens of opportunistic pathogens [3].

There were clear differences in carriage rates for the -308*2 allele of the TNF gene in the various groups. Thus, TNFA-308*2 was carried by similar proportions of HIV-negative controls, non-IRD patients and HCV patients, but was slightly more common in patients with herpesvirus-associated IRD and was not present in any patients who had experienced mycobacterial IRD. We considered whether herpesvirus-associated IRD could be potentiated by genes carried in linkage disequilibrium with TNFA-308*2 in the common European disease-associated MHC haplotype defined by HLA-A1,-B8,-DR3,-DQ2. This was based on the HLA alleles and carriage of allele 2 at a haplotypic marker in intron 10 of the *BAT1* gene, between TNFA-308*2 and HLA-B8 [21]. About 70% of controls and HIV patients with TNFA-308*2 also carry *BAT1*(intron 10)*2 and most of the remainder carry HLA-A2,-B44,-DR4. However, all three patients with a history of CMV retinitis and/or presumptive HSV encephalomyelitis as IRD who had TNFA-308*2 carried HLA-A2,-B44,-DR4 [12] and lacked *BAT1*(intron 10)*2. In contrast, seven of eight non-IRD patients who had TNFA-308*2 carried *BAT1*(intron 10)*2 and HLA-A1,-B8,-DR3 ($P = 0.045$). This highlights the need to consider *TNFA* polymorphisms in the context of the adjacent MHC alleles.

Plasma levels of bioavailable IL-6 increased progressively over 4 years on HAART in patients who experienced CMV retinitis as an IRD [22]. Hence, we investigated a polymorphism at position -174 in the *IL6* gene promoter, which marks increased risk of inflammatory diseases such as juvenile chronic arthritis [14]. Carriage of the C allele at IL6-174 was common in all subgroups, except patients who had IRD associated with mycobacterial infection. When the *IL6* and *TNFA* polymorphisms were considered together, absence of the C allele at IL6-174 in association with TNFA-308*1 conferred a relative risk of 3.0 for mycobacterial IRD. This is interesting because the disease involves mycobacterial replication in affected nodes [4,9,13]. We speculate that the IRD may be facilitated by limited TNF-mediated bacteriocidal activity, as TNFA-308*1 is associated with lower TNFα production in monocytes [23]. Patients with MAC IRD do not exhibit the bacteraemia characteristic of MAC disease in AIDS patients and display restored DTH reactivity to mycobacterial antigens [1,4,13], so the IRD may reflect a T-cell response able to contain but not clear the infection. Perhaps this increases to pathogenic levels in patients homozygous for TNFA-308*1.

The CMV retinitis IRD patients also displayed elevated plasma levels of a type 2 (T2) immune response marker, soluble CD30, before treatment, with progressive increases for 4 years thereafter [5]. Hence, we typed a microsatellite in the CD30 promoter [19] and polymorphisms in the *IL12B* gene [17] which encodes IL12p40, a component of the T1 cytokine, IL-12. The CD30 alleles did not separate this group of patients from patients with IRD associated with infection by other herpesvirus, other opportunistic pathogens or from non-IRD patients. However, 92% of patients who had experienced herpesvirus IRD were homozygous for IL12B-3′UTR*1. This is markedly different from all other patients and suggests dysregulation of the T1/T2 balance in this group of patients.

In conclusion, these results provide indirect evidence of distinct immunopathological mechanisms and inherited susceptibility to IRD associated with mycobacterial or herpesvirus infections. Further study of the role of cytokines in these and other IRD may result in new approaches to their management.

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